Claims

1. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free, protein obtainable from a peripheral membrane fraction of oviductal apical plasma membrane (APM), or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10 kDa and 100 kDa.

2. A method according to claim 1 in which the spermatozoa are contacted with the protein *in vitro*.

3. A method according to claim 1 in which the spermatozoa are boar spermatozoa and the peripheral membrane fraction is of porcine oviductal APM.

4. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 95 kDa.

5. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 60 to approximately 70 kDa.

6. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated,

cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 70 kDa.

7. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 41 kDa.

8. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 38 kDa.

9. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 37 kDa.

10. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 19 to approximately 21 kDa.

11. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane

fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 19 kDa.

12. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 18 kDa.

13. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 13.5 kDa.

14. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, in which the isolated protein comprises ribonucleotideprotein-2.

15. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with ribonucleotideprotein-2 or a fragment or derivative therefrom.

16. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2.

17. A method according to claim 16 wherein said protein includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof.

18. A method of improving and/or prolonging sperm viability following cryopreservation which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa.

19. A method of improving and/or prolonging sperm viability during cryopreservation which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the isolated protein having a molecular weight of between approximately 10kDa and 100kDa.

20. A method of isolating a protein having a molecular weight of between approximately 10kDa and 100kDa, or a fragment or derivative therefrom, having sperm viability improving and/or prolonging activity from oviductal APM comprising the steps of:

(i) harvesting mammalian oviduct epithelial cells;

(ii) separation and isolation of a plasma membrane preparation using a magnesium chloride solution, and centrifugation to obtain a crude APM fraction;

(iii) extraction of a soluble fraction from the crude APM fraction using a salt solution and centrifugation of the solution obtained;

(iv) concentration of the supernatant and washing, to obtain protein.

21. A method according to claim 16 in which the salt solution used in step (iii) is sodium chloride solution.

22. An isolated, cell-free protein having a molecular weight of between approximately 10kDa and 100kDa or a fragment or derivative therefrom, having sperm viability improving and/or prolonging activity, the protein, fragment or derivative obtainable according to the following method:

(i) harvesting mammalian oviduct epithelial cells;
(ii) separation and isolation of a plasma membrane preparation using a magnesium chloride solution, and centrifugation to obtain a crude APM fraction;
(iii) extraction of a soluble fraction from the crude APM fraction using a salt solution and centrifugation of the solution obtained;
(iv) concentration of the supernatant and washing, to obtain protein.

23. A method according to claim 22 in which the salt solution used in step (iii) is sodium chloride solution.

24. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the spermatozoa are microencapsulated.

25. A method according to claim 21 in which the treated spermatozoa are microencapsulated in a semi-permeable membrane comprising poly-lysine.

26. A method of improving and/or prolonging sperm viability comprising contacting spermatozoa with an isolated, cell-

free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the proteins are linked to inert polymers.

27. A method according to claim 26 in which hydrophilic polymers are used.

28. A method according to claim 26 in which the polymer is amine- and carbonyl-reactive dextran.

29. A method for improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the protein is present in a concentration of between approximately 0.1µg/L and approximately 1g/L.

30. A method according to claim 29 in which the protein is present in a concentration of between approximately 5µg/L and approximately 400µg/L.

31. A method according to claim 29 in which the protein is present in a concentration of between approximately 25µg/L and approximately 200µg/L.

32. A method of improving and/or prolonging semen survival following sex-sorting of the spermatozoa for X- (female) and Y-bearing (male) spermatozoa cells which comprises contacting spermatozoa with an isolated protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa.

33. A sperm diluent which includes an additive comprising an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

34. A sperm diluent according to claim 33 in which the diluent or additive is synthetic.

35. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, the protein having a molecular weight of between approximately 10kDa and 100kDa, or a fragment or derivative therefrom, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

36. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 95kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

37. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 60 to approximately 70 kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

38. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight

of approximately 70kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

39. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 41kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

40. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 38kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

41. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 37kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

42. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 19 to approximately 21 kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

43. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 19 kDa, in which the protein, fragment or

derivative has sperm viability improving and/or prolonging activity.

44. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 18 kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

45. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 13.5 kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

46. An isolated, cell free protein, other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2.

47. An isolated, cell free protein according to claim 46 which includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof.

48. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in the manufacture of a composition for improving and/or prolonging sperm viability.

49. Use of ribonucleotideprotein-2 or a fragment or derivative therefrom, in the manufacture of a composition for improving and/or prolonging sperm viability.

50. Use of a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2, in the manufacture of a composition for improving and/or prolonging sperm viability.

51. Use of a protein which includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof, in the manufacture of a composition for improving and/or prolonging sperm viability.

52. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in the manufacture of a composition for improving and/or prolonging sperm viability following cryopreservation.

53. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in the manufacture of a composition for improving and/or prolonging sperm viability during cryopreservation.

54. Spermatozoa together with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity, which are microencapsulated with a semi-permeable membrane.

55. Spermatozoa together with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal

APM, or a fragment or derivative therefrom, the protein comprising ribonucleotideprotein-2, or a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2, or a protein which includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof, and having sperm viability improving and/or prolonging activity, which are microencapsulated with a semi-permeable membrane.

56. A method for identifying an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity the method comprising:

(i) labelling peripheral APM proteins with a marker
(ii) allowing the labelled APM proteins to bind to surface proteins of spermatozoa
(iii) washing to remove excess APM
(iv) adding a detergent to solubilise sperm surface proteins
(v) identifying the isolated proteins labelled with the marker which have bound to the surface proteins of the spermatozoa.

57. An isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity, the protein obtained according to the following method:

(i) labelling peripheral APM proteins with a marker
(ii) allowing the labelled APM proteins to bind to surface proteins of spermatozoa

(iii) washing to remove excess APM

(iv) adding a detergent to solubilise the sperm surface proteins

(v) identifying the APM proteins labelled with the marker which have bound to the surface proteins of the spermatozoa

(vi) separating the labelled APM proteins from the surface proteins of the spermatozoa to obtain isolated protein.